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14. ABSTRACT

Traumatic orthopedic injuries comprise a large portion of the injuries that are seen in our military servicemen in Operation Iraqi Freedom and Operation Enduring Freedom. Previous to this study there had been only one animal model for acute long bone fracture pain described. This model was useful in studying fracture pain by itself, but due to the method of fracture stabilization, the model did not follow real-world circumstance for injury followed by repair. During this period of research we have successfully developed and tested a novel rat pain model for acute traumatic femoral fracture followed by repair via intramedullary nail fixation that closely mimics what happens in real-world situations. Our model is consistent and reproducible and will help facilitate the discovery and evaluation of novel pain relief techniques that could benefit care of our wounded warriors as well as civilian traumatic injuries. We are currently using this model to study the analgesic effect of capsaicin solution applied to the area of the fracture.

15. SUBJECT TERMS
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Introduction

The acute management of pain in the setting of traumatic extremity injury with bone fractures is an integral part in the care of many of the casualties in the current Areas of Responsibility, specifically from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). These injuries are associated with substantial acute pain that is often difficult to treat and may lead to the development of chronic pain syndromes that hamper the wounded soldier's ability to rehabilitate, return to duty, or even to reintegrate into society. The current methods for treating acute pain associated with extremity injuries rely most heavily on opiates given either intravenously or orally. While these agents are useful in the treatment of acute pain, they have serious side effects like respiratory depression and sedation that limit their use especially in forward military locations. In addition, opiate use for pain control can lead to long term opioid tolerance, dependence, and addiction. Most alarmingly, opiate use has been associated with the development of hyperalgesia, leading to a state where the patient may experience pain even at sites distant from the initial injury.[10,15,23,30]

As our ability to adequately treat acute pain is not yet sufficient, even in our modern facilities,[3] much focus has been given to find alternate modalities for acute pain control. Recently, a novel pain receptor site called the transient receptor potential vanilloid type I (TRPV1) has been targeted as a potential site of analgesic therapy. The TRPV1 receptor is a non-selective cation channel that is widely expressed in neuronal tissue and is present on the nociceptive Aδ and C nerve fibers.[34] The TRPV1 channel is important in the development and mediation of neurogenic pain and inflammation, chronic pain, and even may play a role in opioid induced hypersensitivity.[22,33] TRPV1 expression and sensitivity on the cell membrane is modulated by proinflammatory cytokines such as prostaglandin E2 (PGE2), prostacyclin (PGI2), protein kinase C (PKC) and protein kinase A (PKA), as well as by nerve growth factor (NGF) and neurotrophic factor (NTF), among others.[25,28,31]

TRPV1 is activated by the naturally occurring substance, capsaicin, as well as by its analog resiniferatoxin (RTX). Activation of TRPV1 by capsaicin and RTX leads to brief depolarization of the nerve followed by a prolonged inhibition of the receptor leading to blockage of noxious stimulus propagation along sensory nerves and analgesia that may last many weeks[13] while sparing motor and tactile sensory function.[5,20,21,26,27] TRPV1 inactivation has been shown to prevent neurogenic inflammation,[14] and development of distant hyperalgesia induced by inflammation[19] and by nerve injury.[20] Numerous studies have shown the ability of the TRPV1 agonists capcaicin and resiniferatoxin to reduce or eliminate neuropathic pain, hyperalgesia, and pain related to soft tissue injury by either local or perineural infiltration.[17,18,20,21] Additional studies have shown an analgesic benefit in humans using purified capsaicin injection.[7,8,9,29] Moreover, unlike traditional local anesthetics, it seems that both capsaicin and resiniferatoxin have no apparent deleterious effects on nerve tissue[21] or bone healing at low dose.[22]

Recently, lower extremity fracture pain models have been described in two rodent models.[11,24] Both of these models are limited for their use studying the types of injuries incurred in combat as the fracture and surrounding soft tissue injuries are highly modified by having an intramedullary rod in place prior to the fracture. In real world combat injuries, much of the damage produced during the injury is due to deformation and displacement of the bone and subsequent injury to surrounding nerves, vessels, and soft tissues. To our knowledge, there are no published pain models for lower extremity fracture followed by fixation. In addition, there have been no published works evaluating the efficacy of locally applied capsaicin for analgesia in fracture pain or its effects on bone healing and local inflammation.

Body

Approved SOW

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Specific Aim #1 -- Create a rat pain model for lower extremity fracture with open reduction and internal fixation (ORIF).								
Task #1 -- Development of rat lower extremity fracture ORIF model								
1.a -- IACUC approval of protocol	UTHSCSA							
Milestone #1 -- Animal use protocol approval.								
1.b -- Test fracture model and open surgical repair: 10 rats	UTHSCSA	UTHSCSA						
Milestone #2 -- Open fracture repair model achieved								
Task #2 -- Test pain related behaviors in rat fracture model								
2.a -- Fracture and surgical open repair/internal fixation and behavioral testing on 40 Rats		UTHSCSA	UTHSCSA					
2.b -- Sacrifice and Histopathologic/Radiologic study of repaired fracture site		UTSHCA	UTHSCSA	UTHSCSA				
Milestone #3 -- Pain-related behaviors characterized in rat lower extremity ORIF model								
2.c-- Data analysis and manuscript preparation.			All Institutions					
Milestone #4 -- Publication of manuscript on rat pain model for lower extremity fracture with ORIF.								
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**
Specific Aim #2 -- Examine the dose response relationship of capsaicin infiltration to the soft tissue and fracture site to pain, inflammation, and fracture healing								
Task #1 -- Testing of **100** Rats by inducing fracture model and applying different concentrations of capsaicin								
1.a --IACUC protocol approval				UTHSCSA				
1.b --Injure and surgically repair and treat **100** rats followed by behavioral testing.				UTHSCSA	UTHSCSA	UTHSCSA		
1.c --Sacrifice and perform histopathologic and radiologic study of treated rats				UTSHCA	UTSHCA	UTHSCSA	UTHSCSA	
1.d -- Data analysis and interpretation. Evaluate need for additional capsaicin dosing					UTSHCA	UTHSCSA	UTHSCSA	
1.e -- Data analysis and manuscript preparation						All Institutions		
Milestone #5 -- Publication of manuscript on the affect of capsaicin treatment on acute pain, inflammation, and bone healing in our rat model.								

After a brief delay starting work on this research project due to deployment of the PI to Afghanistan, IACUC approval was obtained and work begun in February 2011. The initial work focused on completing Specific Aim #1, developing a traumatic femur fracture repair model in rats. In the original SOW, the intent was to use an open reduction, internal fixation model for fracture repair. However, after discussion with orthopedic surgeons, the PI's own experience at a deployed trauma hospital, and further literature review, it was decided that an intramedullary nail model of fixation was more consistent with the type of repair most commonly used for non-complex femoral fractures in humans and is easily performed in the rat. Subsequently, a model was developed creating a traumatic femoral fracture in a rat followed by repair of the fracture using an anterograde intramedullary nail (Fig 1). This model has been tested, along with control groups, in 30 animals. We have shown that the fracture/repair model is relatively straightforward to accomplish and is highly reproducible. We are in the process of evaluating the fractured femurs by Micro CT scanning and histology for the quality of fracture repair over the 28 day study period. Clinically, all animals appeared to recover completely with no fixation failures.

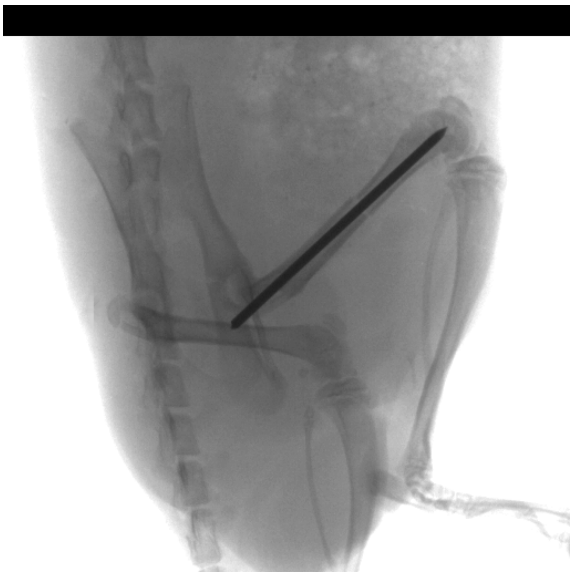

Figure 1: Lateral view of femur fracture with Intramedullary Nail

After successful development of the fracture/repair model, we tested the model for pain behaviors using an incapacitance meter and guarding scores. An incapacitance meter measures the amount of force applied by both hind legs while standing. The amount of force the injured leg applies is reported as a percentage of the total weight from both legs (injured leg force/(left+right leg force)) and represents the amount of weight bearing done by the injured leg. Guarding behavior represents how much the animal protects or guards the injured leg over the course of an hour, with the score reported as a total score over an hour with a higher score reflecting more guarding behavior (favoring of the injured leg). The pain behaviors demonstrated by animals in the fracture/repair group are robust during the initial 2-3 weeks after injury and then return close to baseline compared to non-injured control animals as shown in Figure 2 and Figure 3. This represents a novel animal pain model that has the potential to be useful for the study of the efficacy of different analgesic modalities for acute traumatic fractures.

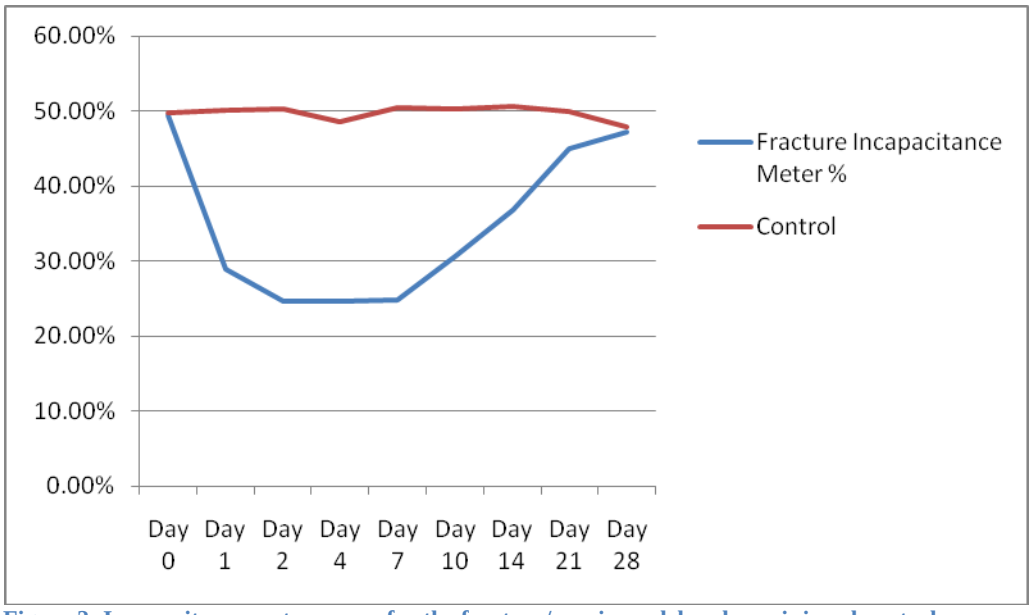

Figure 2: Incapacitance meter scores for the fracture/repair model and non-injured controls.

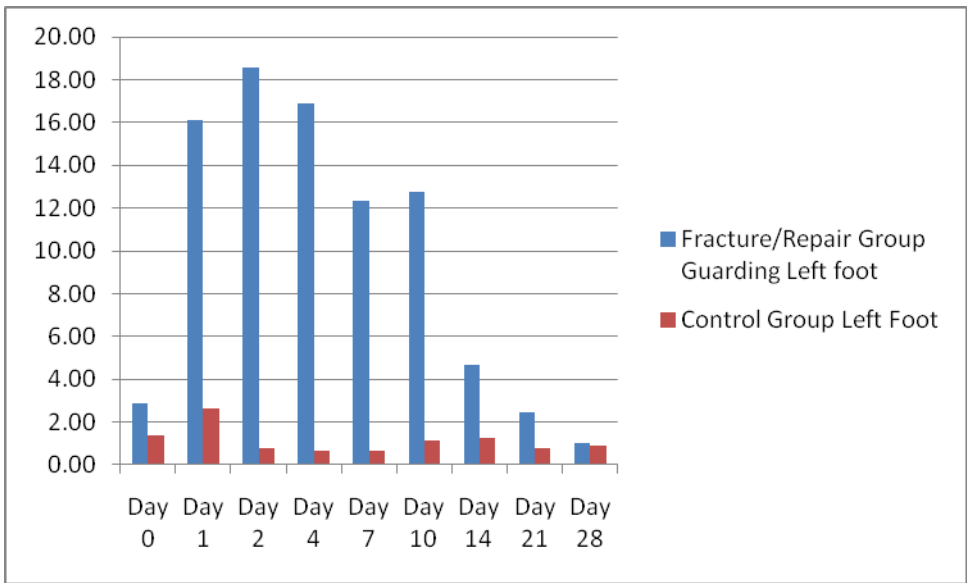

Figure 3: Guarding Behavior Scores of Fracture/Repair and Non-injured Control Groups

After demonstrating robust pain behaviors in a fracture/repair model, we have begun work on Specific Aim #2 to test the efficacy of locally applied capsaicin around the fracture site for analgesia. We have tested ~75% of the animals and preliminary data is promising for showing a reduction in pain behavior in capsaicin treated animals compared to controls. Histology and Micro CT evaluation is ongoing.

Career Development:

The PI has maintained a schedule allowing him at least 2 days/week dedicated to research. During that time, the PI has worked with his mentor, Dr. Brennan, at the University of Iowa to learn methods for animal pain behavior testing, small animal surgical procedures, as well as general study design and execution. The PI has been directly involved in the ongoing research performing all of the surgical procedures and directly overseeing the behavioral testing and care of the animals. In addition, the PI has been able to use his dedicated research time to acquire additional grant funding from the Air Force Surgeon General that funds a study of a novel use of a neuropathic analgesic drug. Because of his research efforts, the PI was named as the Assistant Program Director over Research for the combined Air Force/Army anesthesiology residency program in San Antonio where he mentors residents in training on their research projects. Recently, the PI has been recruited by multiple academic institutions to join their faculty as a researcher in the field of pain medicine.

Key Research Accomplishments

1) Development of a novel closed traumatic femur fracture/repair model
2) Characterization of pain behaviors in the femur fracture/repair model and demonstration of this model as a useful pain model.

Reportable Outcomes

NONE

Conclusion

We have successfully developed a novel traumatic femur fracture/repair pain model in the rat. Pain behaviors were robust with this fracture model and should prove useful in the testing of diverse analgesic modalities for acute fracture pain. It is our belief that this model will help facilitate the development of novel analgesic modalities that will result in better pain relief for traumatic injuries in both our military and civilian populations

Capsaicin treatment and vehicle control groups are at 75% completion. We anticipate completing the pain behavior testing on the capsaicin and control groups by November 2011. Micro CT and histology samples should be complete by early 2012.

References

1. An Y, Friedman RJ, Parent T, Draughn RA. Production of a standard closed fracture in the rat tibia. *J Orthop Trauma* 8 (2004): 687-95.
2. Anand U, Otto WR, Casula MA, Day NC, Davis JB, Bountra C. The effect of neurotrophic factors on morphology, TRPV1 expression and capsaicin responses of cultured human DRG sensory neurons. Neurosci Lett 2006; 339:51-6.
3. Apfelbaum JL, Chan C, Mahta SS, Gan TJ. Postoperative pain experience results from a national survey suggests postoperative pain continues to be under managed. Anesth Analg 2003; 97:534-40
4. Bak B, Jensen KS. Standardization of tibial fractures in the rat. *Bone* 13 (1992): 289-95.
5. Baranowski R, Lynn B, Pini A. The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. Br J Pharmacol 1986;89:267-76.
6. Bonnarens F, Einhorn TA. Production of a standard closed fracture in laboratory animal bone. *J Orthop Res* 2 (1984): 97-101.
7. Cantillon M, Vause E, Sykes D,Tagoe E. Safety, tolerability and efiicacy of intraoperative ALGRX 4975 in a randomized, double-blind, placebo-controlled, study of subjects undergoing bunionectomy. *J Pain* 6 (2005): S48.
8. Davis J, Williams H, Bramlett K, Powell T, Schuster A, Yu KP, Davis J, Gennevois D. A single intra-operative administration of 4975 provides well-tolerated, long-term analgesia for postsurgical pain after total knee arthroplasty. *Pain Med* 10 (2005): 1.
9. Diamond E, Richards PT, Miller T. ALGRX 4975 reduces pain of intermetatarsal neuroma: preliminary results from a randomized, double-blind, placebo-controlled, phase II multicenter clinical trial. *J. Pain* 7 (2006): S41.
10. Doverty M, White JM, Somogyi AA, Bochner F, Ali R, Ling W. Hyperalgesic responses in methadone maintenance patients. *Pain* 90 (2001): 91-96.
11. Freeman KT, Koewler JN, Jimenez-Andrade JM, Buus RJ, Herrera MB, Martin CD, Ghilardi JR, Kuskowski MA, Mantyh PW. A fracture pain model in the rat: Adaptation of a closed femur fracture model to study skeletal pain. *Anesthesiology* 108 (2008): 473-83.
12. Hamalainen MM, Subieta A, Arpey C, Brennan TJ. Differential effect of capsaicin treatment on pain-related behaviors after plantar incision. *J Pain* 10 (Jun 2009): 637-45.
13. Jancso G, Khinyar E. functional linkage between nociception and fluoride-resistant acid phosphatase activing in the Rolando substance. Neurobiology 1975;5:42-3.
14. Jansco G, Kiraly E, Jansco-Gabor A. Direct evidence for an axonal site of action of capsaicin. Naunyn-Schmiedeberg's Arch Pharmacol 1980;313:91-4.
15. King T, Gardell LR, Wang R, Vardanyan A, Ossipov MH. Role of NK-1 neurotransmission in opioid-induced hyperalgesia. *Pain* 116 (2005): 276-88.
16. King T, Vardanyan A, Majuta L, Melemedjian O, Nagle R, Cress AE, Vanderah TW, Lai J, Porreca F. Morphine treatment accelerates sarcoma-induced bone pain, bone loss, and spontaneous fracture in a murine model of bone cancer. *Pain*, no. 132 (2007): 154-68.
17. Kissin I, Bright CA, Bradley EL. Selective and long-lasting nerual blockade with resiniferatoxin prevents inflammatory pain hypersensitivity. *Anesth Analg* 94 (2002): 1253-8.
18. Kissin I, Davison N, Bradley EL. Perineural resiniferatoxin prevents hyperalgesia in a rat mmodel of postoperative pain. *Anesth Analg* 100 (2005): 774-80.
19. Kissin I, Freitas CF, Bradley EL. Memory of Pain: The effect of perineural resiniferatoxin. *Anesth Analg* 103, no. 3 (2006): 721-8.
20. Kissin I, Freitas CF, Bradley EL. Perineural resiniferatoxin prevents the development of hyperalgesia produced by loose ligation of the sciatic nerve in rats. *Anesth Analg* 104, no. 5 (2007): 1210-16.
21. Kissin I, Greitas CF, Mulhern HL, DeGirolami U. Sciatic nerve block with resiniferatoxin: An electron microscopic study of unmyelinated fibers in the rat. *Anesth Analg* 105, no. 3 (2007): 825-31.
22. Kramer SM, May JR, Patrick DJ, Choinard L, Boyer M, Doyle N, Varela A, Smith SY, Longstaff E. Instilled or Injected Purified Natural Capsaicin Has No Adverse Effects on Rat Hindlimb Sensory-Mortor Behavior or Osteotomy Repair. *Anesth Analg* 109 (2009): 24-257.

23. Mao J, Price DD, Mayer DJ. Mechanisms of hyperalgesia and morphine toleranece: A current view of their possible interactions. *Pain*, no. 62 (1995): 259-274.
24. Minville V, Laffosse JM, Fourcade O, Girolami JP, Tack I. Mouse model of fracture pain. *Anesthesiology* 108 (2008): 467-72.
25. Ohta T, Ikemi Y, Murakami M, Imagawa T, Otsuguro KI, Ito S. Potentiation of transient receptor potential V1 functions by the activation of metabotropic 5-hydroxytryptamine receptors in rat primary sensory neurons. J Physiol 2006; 576:809-22.
26. Petsche U, Fleischer E, Lembeck F, Handwerker HO. The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibers. Brain Res 1983;265:233-40.
27. Pini A, Lynn B. Long-term reduction in the number of c-fibre nociceptors following capsaicin treatment of a cutaneous nerve in adult rats. Eur J Neurosci 1990;2:89-97.
28. Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. Nature 2000; 408:985-90.
29. Richards PT, Vasko G, Stasko I, Lacko M, Hewson G. ALGRX 4957 reduces pain of acute lateral epicondylitis: preliminary results from a randomized, double-blind, placebo-controlled, phase II multicenter clinical trial. *J. Pain* 7 (2006): S3.
30. Sjogren P, Honsson T, Jensen NH, Drenck NE, Jensen TS. Hyperalgesia and myoclonus in terminal cancer patients treated with continuous intravenous morphine. *Pain* 62 (1993): 93-97.
31. Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE. Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. J Gen Physiol 2006; 128:509-22.
32. Stoker DG, Gotleib IJ, Comfort S. A single instillatin of a highly purified capsaicin formulatin decreases postoperative pain and analgesic use after bunionectomy surgery: a randomized, double-blind, placebo-controlled study. Pain Med.
33. Vardanyan A, Ruizhong W, Vanderah TW, Ossipov MH, Lai J, Porreca F, King T. TRPV1 receptors in expression of Opiod-induced hyperalgesia. J Pain 2008; 10:243-52.
34. Vennekens R, Owsianik G, Nilius B. Vanilloid Transient Receptor Potential Cation Channels: An Overview. *Curr Pharm Design* 14 (2009): 18-31.